

WHAT IS CLAIMED IS:

1. A hydrophobic lipid-nucleic acid complex consisting essentially of cationic lipids and nucleic acids, said complex being charge-neutralized and soluble in organic solvents.

2. A complex in accordance with claim 1, wherein said nucleic acid is a plasmid.

3. A complex in accordance with claim 1, wherein said cationic lipids are members selected from the group consisting of DODAC, DDAB, DOTMA, DOSPA, DMRIE, DOGS and combinations thereof.

4. A method for the preparation of lipid-nucleic acid particles, comprising:

(a) contacting nucleic acids with a solution comprising non-cationic lipids and a detergent to form a nucleic acid-lipid mixture;

(b) contacting cationic lipids with said nucleic acid-lipid mixture to neutralize the negative charge of said nucleic acids and form a charge-neutralized mixture comprising detergent, nucleic acids and lipids; and

(c) removing said detergent from said charge-neutralized mixture to provide said lipid-nucleic acid particles in which said nucleic acids are protected from degradation.

5. A method in accordance with claim 4, wherein said solution of step (a) further comprises an organic solvent.

6. A method in accordance with claim 4, wherein said cationic lipids are members selected from the group consisting of DODAC, DDAB, DOTMA, DOSPA, DMRIE, DOGS and combinations thereof.

7. A method in accordance with claim 4, wherein said non-cationic lipids are selected from the group consisting of ESM, DOPE, polyethylene glycol-based polymers and combinations thereof.

8. A method in accordance with claim 4, wherein said detergent is octyl- β -D-

glucopyranoside, said cationic lipid is DODAC, said non-cationic lipid is ESM, and said detergent is removed by dialysis.

9. A method in accordance with claim 8, wherein said non-cationic lipids are combinations of ESM and PEG-Ceramide.

10. A method for introducing a nucleic acid into a cell, comprising;

- (a) preparing a lipid-nucleic acid particle according to the method of claim 4; and
- (b) contacting said cell with said lipid-nucleic acid particle for a period of time sufficient to introduce said nucleic acid into said cell.

11. A method in accordance with claim 11, wherein said lipid-nucleic acid particle comprises a plasmid, DODAC and ESM.

12. A method for the preparation of lipid-nucleic acid particles, comprising:

- (a) contacting an amount of cationic lipids with nucleic acids in a solution; said solution comprising of from about 15-35% water and about 65-85% organic solvent and said amount of cationic lipids being sufficient to produce a +/- charge ratio of from about 0.85 to about 2.0, to provide a hydrophobic, charge-neutralized lipid-nucleic acid complex;
- (b) contacting said hydrophobic lipid-nucleic acid complex in solution with non-cationic lipids, to provide a lipid-nucleic acid mixture; and
- (c) removing said organic solvents from said mixture to provide said lipid-nucleic acid particles in which said nucleic acids are protected from degradation.

13. A method in accordance with claim 12, wherein said cationic lipids are members selected from the group consisting of DODAC, DDAB, DOTMA, DOSPA, DMRIE, DOGS and combinations thereof.

14. A method in accordance with claim 12, wherein said non-cationic lipids are members selected from the group consisting of ESM, DOPE, polyethylene glycol-based polymers and combinations thereof.

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15. A method in accordance with claim 12, wherein said organic solvents are members selected from the group consisting of methanol, chloroform, methylene chloride, ethanol, diethyl ether and combinations thereof.

16. A method in accordance with claim 12, wherein said nucleic acid is a plasmid, said cationic lipid is a member selected from the group consisting of DODAC, DDAB, DOTMA, DOSPA, DMRIE, DOGS and combinations thereof, said non-cationic lipid is a member selected from the group consisting of ESM, DOPE, polyethylene glycol-based polymers and combinations thereof, and said organic solvent is a member selected from the group consisting of methanol, chloroform, methylene chloride, ethanol, diethyl ether and combinations thereof.

17. A method for introducing a nucleic acid into a cell, comprising;

- (a) preparing a lipid-nucleic acid particle according to the method of claim 13; and
- (b) contacting said cell with said lipid-nucleic acid particle for a period of time sufficient to introduce said nucleic acid into said cell.

18. A lipid-nucleic acid particle prepared according to claim 4.

19. A lipid-nucleic acid particle prepared according to claim 12.

20. A method for the preparation of serum-stable plasmid-lipid particles, comprising:

- (a) combining a plasmid with cationic lipids in a detergent solution to provide a coated plasmid-lipid complex;
- (b) contacting non-cationic lipids with said coated plasmid-lipid complex to provide a solution comprising detergent, a plasmid-lipid complex and non-cationic lipids; and
- (c) removing said detergent from said solution of step (b) to provide a solution of serum-stable plasmid-lipid particles, wherein said plasmid is encapsulated in a lipid bilayer and said particles are serum-stable and have a size of from about 50 to about 150 nm.

21. A method in accordance with claim 20, wherein said removing is by dialysis.

22. A method in accordance with claim 20, wherein step (b) further comprises adding a polyethylene glycol-lipid conjugate.

23. A method in accordance with claim 22, wherein said polyethylene glycol-lipid conjugate is a PEG-ceramide conjugate.

24. A method in accordance with claim 20, further comprising;
(d) sizing said particles to achieve a uniform particle size.

25. A method in accordance with claim 20, wherein said cationic lipids are selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol and combinations thereof.

26. A method in accordance with claim 20, wherein said non-cationic lipids are selected from the group consisting of DOPE, POPC, EPC and combinations thereof.

27. A method in accordance with claim 20, wherein said detergent solution comprises a detergent having a critical micelle concentration of between about 20 mM and 50 mM.

28. A method in accordance with claim 8, wherein said detergent is n-octyl- β -D-glucopyranoside.

29. A method for the preparation of serum-stable plasmid-lipid particles, comprising;

- (a) preparing a mixture comprising cationic lipids and non-cationic lipids in an organic solvent;
- (b) contacting an aqueous solution of plasmid with said mixture prepared in step (a) to provide a clear single phase; and
- (c) removing said organic solvent to provide a suspension of plasmid-lipid particles, wherein said plasmid is encapsulated in a lipid bilayer, and said

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particles are stable in serum and have a size of from about 50 to about 150 nm.

30. A method in accordance with claim 29, wherein said non-cationic lipids comprise a polyethylene glycol-lipid conjugate.

31. A method in accordance with claim 30, wherein said polyethylene glycol-lipid conjugate is a PEG-ceramide conjugate.

32. A method in accordance with claim 29, wherein said cationic lipids are selected from the group consisting of DODAC, DDAB, DOTAP, DOTMA, DOSPA, DOGS, DC-Chol and combinations thereof.

33. A method in accordance with claim 29, wherein said non-cationic lipids are selected from the group consisting of DOPE, POPC, EPC and combinations thereof.

34. A plasmid-lipid particle prepared according to claim 20.

35. A method for introducing a plasmid into a cell, comprising;

- (a) preparing a plasmid-lipid particle according to the method of claim 20; and
- (b) contacting said cell with said plasmid-lipid particle for a period of time sufficient to introduce said plasmid into said cell.

36. A method in accordance with claim 35, wherein said plasmid-lipid particle comprises a plasmid, DODAC, POPC and a PEG-Ceramide selected from the group consisting of PEG-Cer-C₂₀ and PEG-Cer-C₁₄.

37. A method in accordance with claim 35, wherein said plasmid-lipid particle comprises a plasmid, DODAC, DOPE and a PEG-Ceramide selected from the group consisting of PEG-Cer-C₂₀ and PEG-Cer-C₁₄.

38. A plasmid-lipid particle prepared according to claim 29.

39. A method for introducing a plasmid into a cell, comprising;

- (a) preparing a plasmid-lipid particle according to the method of claim 29; and
- (b) contacting said cell with said plasmid-lipid particle for a period of time sufficient to introduce said plasmid into said cell.

40. A method in accordance with claim 39, wherein said plasmid-lipid particle comprises a plasmid, DODAC, POPC and a PEG-Ceramide selected from the group consisting of PEG-Cer-C₂₀ and PEG-Cer-C₁₄.

41. A method in accordance with claim 39, wherein said plasmid-lipid particle comprises a plasmid, DODAC, DOPE and a PEG-Ceramide selected from the group consisting of PEG-Cer-C₂₀ and PEG-Cer-C₁₄.

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